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FURTHER STUDIES ON STREPTOCOCCUS INFECTIONS.*†

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ABOUT two years ago Dr. Weaver and I[1] showed that the blood serum from scarlet-fever patients has no streptococcidal power *in vitro*, in any stage of the disease. Through the researches of Lemoine, Slawyk, Hektoen, and others we know that the streptococci are occasionally found circulating in the blood of these patients, and that the patients in whose blood the cocci are found often make an uneventful recovery. Jochmann,[2] however, is of the opinion that the prognosis is very bad when streptococci are found in the blood. What is true of scarlet fever may equally well be said of tonsillitis. Here, too, streptococci may invade the blood stream, but in spite of this fact the patient may make a rapid and satisfactory recovery.[3] In erysipelas and in many wound and puerperal infections the tissues are invaded by virulent streptococci, but we know that in a great majority of these cases the organisms disappear sooner or later, and the patients make a complete recovery.

We know that normal human serum has no streptococcidal power. Hence the disappearance of cocci from the blood and tissues cannot be explained by assuming that the serum lacks something during the height of the disease, but regains this substance as convalescence sets in, and thus destroys the cocci. It is evident, therefore, that we must look for some other agent than the serum alone to account for the disappearance of the cocci from the blood and tissues during convalescence. According to Metchnikoff, Denys and Leclef, Bordet, Marchand, and others,[4] this agent is found in the phagocytes. This view has not been universally accepted, although experiments show

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[1] *Trans. Chicago Path. Soc.*, 1903, 5, p. 285. *Medicine*, 1903, 9; p. 515.

[2] *Deutsches Arch. f. klin. Med.*, 1903, 5, p. 209.

[3] ROSENOW, *Am. Jour. of Obstetrics*, 1904, 50, p. 766.

[4] For a more complete review of the literature on phagocytosis see *Jour. A. M. A.*, 1905, 44, p. 198.

that it explains the facts better than any other theory that has been advanced. In a previous paper[1] I was able to show that the leucocytes are the most important, if not the only, factor concerned in the destruction of streptococci in the body of infected rabbits and guinea-pigs. The view was there expressed that in man also the leucocytes may play an important rôle in combating infection by these organisms, although not much work had been done on human infections.

In this paper are set forth the results of further studies of streptococcus infections in human subjects, in the hope of determining just what factors are concerned in the destruction of the invading cocci; and an attempt is made to analyze more fully this phenomenon. In all instances where blood was used, it was drawn from the vein at the elbow by means of a Luer syringe, and defibrinated by gently whipping with a sterile wire. If the defibrinating is carefully performed, not a very large proportion of the leucocytes are destroyed. In the experiments each tube contained 0.8–1.0 c.c. of blood or serum, which was inoculated with one loopful of streptococcus culture, and two loopfuls from each tube were plated in glucose agar at intervals. The tubes were always kept in the incubator at 36°C. When highly virulent organisms were used, 0.3–0.4 c.c. of defibrinated rabbit blood was added to each tube of melted agar to facilitate the counting of colonies which are often very small if no blood has been added.

Table 1 shows that, although human serum, *in vitro*, is a good culture medium for streptococci, normal defibrinated blood has a slight streptococcidal power. Occasionally we may find a sample of normal blood which destroys many non-virulent streptococci, but the virulent organisms usually multiply in this blood. Table 2 shows that defibrinated blood from patients suffering from an acute infection has a much greater destructive effect upon these organisms than has normal blood. In these infections the leucocyte count is usually somewhat increased, and it seems that the streptococcidal power of the blood, *in vitro*, is roughly proportional to the leucocyte count. That is, the higher the leucocyte count, the greater will be the streptococcidal power of the blood. This is a general rule, to which there are, however, a few exceptions, to be pointed out later. The virulent organisms frequently multiply in these bloods unless the leucocytosis is quite high. In no instance could a streptococcidal power of the serum alone be detected. It might be objected that we are not dealing here with an actual destruction of cocci, but that the decrease in the number of colonies on the plates is due to adherence of the cocci to the leucocytes. This objection is ruled out by the fact that the 24 hour plates from tubes containing blood with a high leucocytosis are very often sterile, or nearly so.

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[1] *Jour. A. M. A.*, 1905, 44, p. 198.

It has been thought possible that the serum, during the course of an infection which terminates favorably, might acquire streptococcidal properties for that particular race of streptococci which is responsible for the infection, while at the same time it had no such properties for other races of these organisms. Three strains of streptococcus were therefore isolated from erysipelas patients, and the serum of each patient tested on the corresponding organism. All of the patients made a satisfactory recovery, but at no time could streptococcidal properties be demonstrated in their serum. The defibrinated blood, on the other hand, killed many cocci, while there was an increased leucocytosis.

We know that there is an intense local reaction in the localized streptococcus infections, and it has been thought by some that there may be lysis of cocci by the inflammatory serum in these areas. This

TABLE I.

THE EFFECT OF DEFIBRINATED NORMAL HUMAN BLOOD AND HUMAN SERUM UPON STREPTOCOCCI.

STREPTOCOCCI	DEFIBRINATED BLOOD	COLONIES ON AGAR PLATES		
		Immediate	2-3 Hours	5 Hours
300*	I	1,100	....	160
300	II	3,000	....	1,060
300..............	IIa	680	675	1,300
300	III	2,600	2,700	3,500
300	IV	3,000	1,050	2,600
300	V	540	360	240
300	VI	1,100	500	390
298	VII	1,600	600	315
298..............	VIII	600	80	16
B104.............	IIa	76	55	510
B104.............	III	1,800	2,800	Many
B104.............	IV	1,600	3,000	“
B104.............	V	500	600	900
381	VI	2,000	2,300	Many
300	Serum VI	1,400	2,000	“
298	Serum VII	1,700	2,500	10,000

* Streptococcus 300 and 298 are non-virulent, as they have been kept on artificial media for two years. B104 and 381 have been passed through many rabbits and are highly virulent.

theory is difficult to confirm or refute, on account of the difficulty of obtaining inflammatory serum in the same condition as that in which it is found in the tissues. As it is not uncommon to find blebs of considerable size on the affected parts of erysipelas patients, the fluid from these blebs was taken as the nearest approximation to the inflammatory serum. This blister fluid, from several cases of erysipelas, was tested for streptococcidal properties, but gave negative results.

TABLE 2.

THE EFFECT OF DEFIBRINATED BLOOD FROM CASES OF SCARLATINA, ERYSIPELAS, TONSILLITIS, AND PNEUMONIA UPON STREPTOCOCCI.

STREPTOCOCCI	DEFIBRINATED BLOOD	COLONIES IN GLUCOSE AGAR PLATES			
		Leucocyte Count	Immediate	2–3 Hours	5 Hours
300	Scarlatina I	10,500	1,100	....	6
300	" II	10,600	2,000	....	140
300	" III	15,000	690	270	116
300	" IV	.....	550	18	3
300.	" V	.....	1,500	300	150
300	" VI	.....	1,900	350	43
300	" VII	.....	500	32	12
300	" VIII	13,000	1,380	102	9
300.	" IX	.....	1,200	125	14
300	" X	13,400	1,250	245	120
300.	" IX	.....	1,950	150	19
300	" XII	10.800	1,800	700	130
300	" XIII	.....	1,800	300	45
300	" XIV	12,300	1,500	31	2
381	" III	15,000	1,200	1,500	3,500
B104.......	" IV	.....	600	462	415
B104.......	" V	.....	1,800	1,800	5,000
381	" VI	.....	220	51	1,100
381.	" VII	.....	130	41	Many
381.	" VIII	13,000	360	1,180	"
381.......	" IX	13,000	2,200	1,950	"
B104.......	" IX	.....	330	190	1,500
381	" X	13,400	650	290	2,500
B104.......	" X	.....	400	160	165
381	" X	.....	320	650	Many
381	" XII	10,800	200	2,000	"
381	" XIII	.....	540	900	"
300	Erysipelas I	.....	630	64	0
300	" II	15,000	800	14	4
300	" IIa	.....	1,100	41	8
300	" III	.....	740	420	160
300	" IV	.....	1.650	480	150
298	" V	10,400	3,100	420	92
B104.......	" I	.....	1,200	1,080	960
B104.......	" II	.....	96	30	13
B104.......	" IV	.....	133	110	480
300	Tonsillitis I	16,000	620	34	2
B104.......	" I	.....	300	59	70
300	" II	.....	1,400	74	6
300	Pneumonia I	.....	2,600	0	3
300	" II	.....	00	5	0
300	Ser. scar. VII	.....	1,500	3,500	10,000
300	" XIV	.....	1,200	1,400	1,300
300	Erysipelas II	.....	670	760	750
300	" III	.....	700	1,800	6,000
300	" V	.....	3,000	3,500	5,000
B104.......	" V	.....	190	360	1,140

The importance of a high leucocyte count in the destruction of streptococci by blood is clearly shown by the accompanying record of experiments.

Experiment 1.—Ten c.c. of blood were drawn from the vein at the elbow of an erysipelas patient, and carefully defibrinated. One c.c. of the defibrinated blood, which contained 9,800 white corpuscles per cubic millimeter, was put into small test tubes, inoculated with one loopful of virulent streptococcus culture, and two loopfuls of the inocculated blood plated at intervals. The remaining 8 c.c. were centrifugated and most of the serum was drawn off. We know that the uppermost stratum of centrifugated corpuscles contains a high percentage of leucocytes, because they are thrown down less easily than the red corpuscles. This stratum was therefore drawn off with a sterile pipette, and mixed with a small quantity of serum. The resultant mixture contained 17,200 leucocytes per cubic millimeter. One c.c. of this "suspension of leucocytes" was introduced in a small test tube, inoculated, and plates were made as before. To complete the experiment, 1 c.c. of the clear serum was put into a small tube, which was likewise inoculated, and plates were made at intervals. This experiment was also performed with normal blood and a non-virulent streptococcus. The plates were incubated for 24 hours, the colonies that developed on each were counted, with the results shown in Table 3.

TABLE 3.

STREPTOCOCCUS		LEUCOCYTE COUNT	COLONIES ON AGAR PLATES		
			Immediate	2–3 Hours	5 Hours
B104	Erysipelas blood	9,800	390	168	350
B104	Suspension erysipelas leucocytes	17,000	260	58	21
B104	Erysipelas serum	....	200	360	1,100
298	Normal blood	4,500	1,650	600	316
298	Suspension normal leucocytes	6,600	1,600	420	62
298	Normal serum	....	1,700	2,500	00

The table shows that both strains of streptococcus which were used multiplied in the cell-free serum; that the defibrinated blood destroyed many of the non-virulent and some of the virulent cocci; and that the " suspension of leucocytes " destroyed more cocci of either strain than did the defibrinated blood. The only difference between the defibrinated blood and the suspension of the leucocytes lay in the fact that the latter contained nearly twice as many leucocytes as the former.

The fact that the streptococcidal power of the blood is dependent upon its number of leucocytes per cubic millimeter has also been demonstrated by a second experiment.

Experiment 2.— Shortly after a patient's admission into the hospital 3 c.c. of blood were drawn from a vein at the elbow and defibrinated. The leucocyte count at this time was 11,000. The blood was divided equally among three tubes, each tube was inoculated with streptococcus culture, and two loopfuls from each were plated at intervals. Shortly after drawing the blood, the patient was injected under the skin of the back with 10 c.c. of an antistreptococcus serum. This brought about an increase in the leucocytosis up to 15,000* five hours after the injection. Three c. c. of blood were again drawn from the vein at the elbow, and its effect on streptococci was tested as before. The results of the experiment are shown in Table 4.

TABLE 4.

STREPTOCOCCUS		LEUCOCYTOSIS	COLONIES ON AGAR PLATES		
			Immediate	2–3 Hours	5 Hours
300........	Blood before injection	11,000	1,100	360	200
B104.......	" " "	.....	390	380	4,000
381........	" " "	.....	100	500	Many
300........	Blood 5 hrs. after injection	1,500	1,300	44	0
B104.......	" 5 " " "	.....	412	246	600
381........	" 5 " " "	.....	150	270	3,000

The blood drawn after the injection of the serum, when the leucocytosis was high, has a greater streptococcidal power than that drawn before the injection. It would not be safe to conclude that this difference in the streptococcidal powers is due entirely to the difference in the leucocyte count. Some of it might be due to an antitoxic or opsonic action of the injected serum, or to a stimulation of the leucocytes. This supposition loses most of its force when we consider the fact that the addition of 1 to 5 per cent of antistreptococcus serum to defibrinated blood, *in vitro*, does not increase its streptococcidal power, as shown by Dr. Hektoen and myself.

Wright and Douglas[1] have shown that phagocytosis takes place only after the bacteria have been sensitized, that is, have been acted upon by the opsonin of the serum. There is no phagocytosis of a suspension of washed leucocytes in NaCl solution, or in heated serum, regardless of the number of untreated bacteria that are added. This work has been confirmed and extended by Hektoen and Ruediger,[2] and by Bulloch and Atkin.[3] In view of these facts, there should be no reduction in the number of streptococci in a test tube containing a suspension of washed corpuscles in salt solution or in heated serum, and the following experiment shows that such is the case.

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* I am indebted to Dr. Tunnicliff for the leucocyte counts and the injection of the serum.

[1] *Proc. Roy. Soc.*, 1903, 72, p. 357, and 1904, 73, p. 128.

[2] *Jour. Infect. Dis.*, 1905, 2, p. 128.

[3] *Proc. Roy. Soc.*, 1905, 74, p. 379.

Experiment 3.—Ten c.c. of blood were drawn from a vein at the elbow of a scarlet-fever patient, defibrinated, centrifugated, and the serum drawn off. The corpuscles were washed twice in a large amount of NaCl solution and 0.5 c.c. of the centrifugated corpuscles placed into each of three small tubes containing 0.5 c.c. of normal serum, 0.5 c.c. of heated (58° for one-half hour), and 0.5 c.c. of salt solution, respectively. The tubes were inoculated with one loopful of streptococcus culture, and two loopfuls from each were plated at intervals, with the results shown in Table 5:

TABLE 5.

STREPTOCOCCUS		COLONIES ON GLUCOSE AGAR PLATES		
		Immediate	5 Hours	24 Hours
300........	Washed corpuscles + serum	1,500	7	21
300........	" " + heated serum	1,400	5,000	Many
300........	" " + NaCl solution	1,700	3,500	"
300........	Serum	1,200	1,400	"

The importance of opsonin in the destruction of streptococci is further shown by the fact that the defibrinated blood from two obstinate cases of post-scarlatinal nephritis had no streptococcidal powers, although in one of these cases the leucocyte count was 14,000. When the blood from these patients was centrifugated, and the corpuscles suspended in serum from a patient who was convalescent and had no nephritis, the resultant suspension had a small degree of streptococcidal power, as shown by Table 6:

TABLE 6.

STREPTOCOCCUS		COLONIES ON AGAR PLATES		
		Immed.	2 Hours	5 Hours
298........	"Convalescent blood"	800	450	14
298........	"Nephritis blood"	720	1,900	6,000
298........	"Nephritis corpuscles" + "conv. ser."	850	500	540
298........	"Conv. washed corp." + "neph. ser."	725	600	61

The interesting fact came to light in these experiments that the combination "convalescent washed corpuscles" + "nephritis serum" has nearly as great a streptococcidal power as the defibrinated blood from the patients without nephritis. The corpuscles were washed twice in a large amount of NaCl solution, which is usually sufficient to prevent phagocytosis in a suspension of corpuscles in NaCl solution. It is not likely, therefore, that the washing had not been carried far enough. But the results of these experiments seem to indicate rather that the leucocytes, as well as the serum, from these nephritis patients have undergone some change which renders them less efficient in the destruction of bacteria. In fact, it would seem that the leucocytes have suffered more than the serum. Whether or not these facts may serve to throw light on the cause of some of the terminal infections cannot be determined at this time.

Whether or not the opsonin is increased during the acute infections is an interesting question. Normal leucocytes in normal serum take up large numbers of cocci; hence it is difficult to determine if leucocytes in erysipelas serum, for instance, take up more cocci than those in normal serum. This question had, therefore, to be approached in a different way. Two sets of tubes were made, and 0.2 c.c. of washed corpuscles introduced into each. To one set of tubes were added falling quantities of normal serum, and to the other set falling quantities of erysipelas serum. The contents of each tube were made up to 0.4 c.c. with NaCl solution, and to each tube was added 0.4 c.c. of a suspension of streptococci. The tubes were incubated for one hour at 36° C., smears made, and the average number of cocci in each leucocyte determined by counting those in 30 leucocytes. The results are shown in Table 7:

TABLE 7.

Normal Serum	Phagocytosis	Erysipelas Serum	Phagocytosis
0.2	13.5	0.2	11.5
0.1	9.4	1.1	11.2
0.05	6.7	0.05	8.2
0.025	4.7	0.025	6.9
0.012	2.2	0.012	4.3

This experiment indicates that there is a slight increase of opsonin in the erysipelas serum as compared with normal serum. A similar increase of opsonin has been noted by Wright and Douglas[1] after treating a person afflicted with furunculosis with their staphylococcus vaccine. A diminution of opsonin in persons subject to attacks of furunculosis, sycosis, etc., has also been observed by these investigators.

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[1] *Proc. Roy. Soc.*, 1904, 73, p. 128.

CONCLUSIONS.

Human serum does not acquire streptococcidal properties during the course of a streptococcus infection.

The blister fluid from erysipelas patients has no streptococcidal powers.

Defibrinated human blood has a streptococcidal power, which, with few exceptions, is roughly proportional to the leucocytosis.

The destruction of cocci in the defibrinated blood is brought about by the leucocytes, but, before this can be accomplished, the cocci must be acted on by the opsonin of the serum. There is no phagocytosis, and hence no destruction of unsensitized cocci by washed leucocytes.

The opsonin is increased during the course of an attack of erysipelas.

In an attack of acute nephritis the opsonin is diminished, but the leucocytes also undergo a change which renders them less effective in the destruction of streptococci. This fact may serve to throw light on the cause of many terminal infections.

I wish to thank Professor Hektoen for many suggestions, and the internes at Cook County Hospital for many courtesies.